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# 大花沿阶草中的甾体皂甙成分

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**摘要** 从大花沿阶草(*Ophiopogon maglanthus* Wang et Dai)的鲜根中分离到4个甾体皂甙,根据详细的化学和光谱分析,它们分别被证明为 prosapogenin A of diocin, deltonin, *O. planiscapus* 中的 glycoside D 和 glycoside F.

**关键词** 大花沿阶草; 百合科; 甾体皂甙

# STEROIDAL SAPONINS FROM OPHIOPOGON MAGLANTHUS

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**Abstract** Four steroidal saponins were isolated from the fresh roots of *Ophiopogon maglanthus*. On the basis of chemical and spectroscopic analysis, they were proved to be identical with prosapogenin A of diocin, deltonin, glycosied D and glycosied F, respectively. These saponins had ever been obtained from other species of *Ophiopogon*.

**Key words** *Ophiopogon maglanthus*; Liliaceae; Steroidal saponin

## INTRODUCTION

The genus *Ophiopogon* (Liliaceae) with more than 60 species is concentrated in the tropical and subtropical regions of eastern and southern Asia. Many species which have been used as traditional medicines in China have already been studied chemically and were found to contain steroidal saponins [1—5]. As a part of our continuing phytochemical investigation of Liliiflorae plants for providing evidence of chemotaxonomy, we now have examined the methanol extract of *O. maglanthus* Wang et Dai, a species from south Yunnan. The results are present in this paper.

## RESULTS AND DISCUSSION

Saponins 1—4 were obtained by repeated CC on silica gel in yields of 0.037, 0.041,

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0.185 and 0.55% of the fresh roots, respectively.

Saponin 1 was positive in the Liebermann–Burchard reaction, and it showed strong absorption of hydroxyl groups and characteristic absorption bands of a 25 (R) –spirostanol moiety in IR spectrum [6] . The FAB mass spectrum of 1 exhibited molecular ion peaks at m / z 745 $[M(C_{39}H_{62}O_{12})+Na]^+$and 729 $[M+Li]^+$. On acid hydrolysis on TLC plate [7] , 1 afforded D–glucose and L–rhamnose in a ratio of 1: 1 as sugar residues. The EI mass spectrum of its acetate showed fragment ions at 273 [terminal $rha(Ac)_3]^+$and 561$[rha(Ac)_3+glu(Ac)_3]^+$. The $^{13}C$ and $^1H$ NMR spectra of 1 revealed that it was a 3–O–glycoside of diosgenin [8] with a terminal α–L–rhamnopyranosyl unit and an inner β–D–glucopyranosyl unit [anomeric carbons: δ 101.9, 100.4; anomeric protons: δ 6.302 (1H, s), 4.980 (1H, d, J = 7.2 Hz)]. In the $^{13}C$ NMR spectrum, it was observed that the signal due to C–2 of the D–glucose was displaced downfield at δ 79.4. Therefore, the terminal L–rhamnose was attached to the hydroxy group at C–2 of the inner D–glucose. From these results, 1 was concluded to be diosgenin –3–O–α–L–rhamnopyranosyl (1→2)–β–D–glucopyranoside, and it was identical with prosapogenin A of diocin by comparison of physical and spectral data with an authentic sample [2, 3] .

Saponin 2, $C_{45}H_{72}O_{17}$, gave D–glucose and L–rhamnose in a raio of 2:1 as sugar components on acid hydrolysis on TLC plate. The $^{13}C$ and $^1H$ NMR spectra of 2 revealed that it was a diosgenin trioside [8] . The fragment ions at m / z 273 and 331 in the EI mass spectrum of its acetate indicated the presence of a terminal rhamnose and a terminal glucose. Partial hydrolysis of 2 with 0.5 N HCl in 50% aq. ethanol yielded a prosapogenin (2a). Based on the analysis of $^{13}C$ NMR spectrum, 2a was identified as diosgenin–3–O–β–glucopyranosyl (1→4)–β–D–glucopyranoside [3] . Finnally, saponin 2 was determined as diosgenin–3–O–α–L–rhamnopyranosyl (1→ 2)–[ β–D–glucopyranosyl (1→4)]–β–D–glucopyranoside by comparison of $^{13}C$ NMR spectra and physical date with an authentic sample [3] , and it was identical to deltonin reported by Passeshnichenko et al [9] .

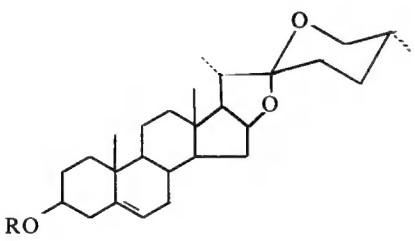
Saponin 3 was a mixture of two compounds with close Rf values. When the TLC was sprayed with 10% aq. $H_2SO_4$, followed by heating, both glycosides were revealed in the form of red spots, while the use of Ehrlich reagent [10] have pink–red spots. There were no any characteristic spirostanol absorption bands in the IR spetrum. This suggested that 3 was a pair of furostanol saponins. When the combined saponins were refluxed in aq. acetone and methanol, the individual saponins 3a (22–OH) and 3b (22–$OCH_3$), respectively, were obtained.

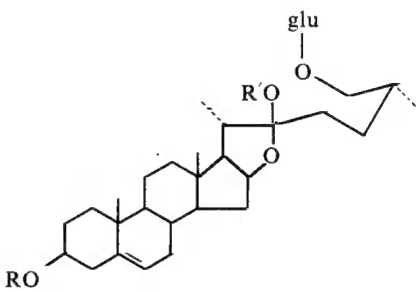
Generally [11, 12] , the complete acid hydrolysis of the furostan glycosides forms the corresponding sapongenin, while enzymatic hydrolysis forms glycosides of the spirostan sreies with the loss of one D–glucose . As a rule, the latter is a attached to the hydroxy

group at C–26 of the aglycone.

On enzymatic hydolysis with cellulase, the combined saponins 3 afforded a prosapogenin and D–glucose. This prosapogenin was proved to be identical with 1. Accordingly, 3 was established as 26–$\beta$–D–glucopyranosyl 22–hydroxy (methoxy) –25(R)–furost–5–ene–3$\beta$, 26–diol–3–O–$\alpha$–L–rhamnopyranosyl (1→2)–$\beta$–D– glucopyranoside on the basis of $^{13}C$ NMR spectrum of 3a. As a natural , saponin 3a was previously obtained from *O. planiscapus* and named glycoside D [3] .

Analogous to saponin 3, 4 was a combined saponins. When 4 was treated as above, the individul saponins 4a ($OCH_3$) and 4b (OH), respectively, were obtained. Enzymatic hydrlysis of 4 gave 2 and 1 as prosapogenins. Based on the analysis of $^{13}C$ NMR spectra of 4a and 4b, the structure of 4 was elucidated to be 26–$\beta$–D–glucopyranosyl 22–hydroxy (methoxy)–25(R)–furost–5–ene–3$\beta$, 26–diol–3–O–$\alpha$–L–rhamnopyranosyl (1→2)–[ $\beta$–D–glucopyranosyl (1→4)–$\beta$–D–glucopyranoside, and 4b was identical to glycoside F which was also isolated from *O. planiscapus* [3] .





| | R |
|---|---|
| 1 | –glu(2→)rha |
| 2 | –glu(2→)rha, glu(4→) |
| 2a | –glu(4→)glu |

| | R | R′ |
|---|---|---|
| 3a | –glu(2→)rha | –H |
| 3b | –glu(2→)rha | –$CH_3$ |
| 4a | –glu(2→)rha, glu(4→) | –$CH_3$ |
| 4b | –glu(2→)rha, glu(4→) | –H |

glu: $\beta$–D–glucopyranosyl; rha: $\alpha$–L–rhamnopyranosyl

## EXPERIMENTAL

Mps: uncorr. Optical rotations were measured in pyridine. $^1H$ and $^{13}C$ NMR spectra were recorded in pyridine–$d_5$ with Bruker AM–400 spectrometer, using TMS as

int. std. FABMS were taken on a ZAB–HS spectrometer. EIMS were measured at 20 eV accelerating votage after micro–scale acetylation.

**Plant material.** Roots of *O. maglanthus* Wang et Dai were collected in Fadou, Xichou, Yunnan province, China, and identified by Prof. H. Li and Mr. Y. P. Yang. A voucher specimem has been deposited in the Herbarium of Kunming Institute of Botany.

**Extraction and isolation.** The fresh roots (488 g) were extracted with hot MeOH, then concd in vacuo. The resulting residue (57 g) was dissolved in $H_2O$, extd with $Et_2O$ followed with n–BuOH (sat. with $H_2O$). The combined n–BuOH layers were concd under reduced presure to afford a yellow powder (10.5 g), which was subjected to CC on silica gel with $CHCl_3$–MeOH–$H_2O$ (50: 10: 1 to 10: 10: 1) to provide frs 1–5.

Fr. 2 and Fr. 3 were recrystallized from MeOH to give 1 (180 mg) and 2 (200 mg), respectively.

Saponin 1. Colourless pellets, mp 200–223℃, $[\alpha]_D^{21}$–95.1° (pyridine; c 0.497); IR $\nu_{max}^{KBr}cm^{-1}$: 3440–3380, 980, 920, 900, 865 (intensity 920 < 900, 25(R) spirostanol); FABMS m/z: 745 $[M(C_{39}H_{62}O_{12})+Na]^+$, 729 $[M+Li]^+$; EIMS (acetate) m/z: 273$[rha(Ac)_3]^+$, 561$[rha(Ac)_3+glu(Ac)_3]^+$; $^1H$ NMR$\delta$: 0.710 (3H, d, J = 5.3 Hz, $CH_3$), 0.834 (3H, s, $CH_3$), 1.045 (3H, s, $CH_3$), 1.144 (3H, d, J = 7.8 Hz, $CH_3$), 1.739 (3H, d, J = 6.2 Hz, rha–$CH_3$), 4.980 (1H, d, J = 7.2 Hz, glu–$H_1$), 5.315 (1H, m, olefinic H), 6.302 (1H, s, rha–$H_1$); $^{13}C$ NMR: Tables 1–2.

Saponin 2. colourless needles, mp 293—295℃, $[\alpha]_D^{21}$–93.1° (pyridine; c 0.29); IR $\nu_{max}^{KBr}cm^{-1}$: 3460—3420, 980, 920, 900, 865 (intensity 920 < 900, 25(R) spirostanol); FABMS m/z: 907 $[M(C_{45}H_{72}O_{17})+Na]^+$, 891 $[M+Li]$; EIMS (acetate) m/z: 273 $[rha(Ac)_3]^+$, 331 $[glu(Ac)_4]^+$; $^1H$ NMR$\delta$: 0.705 (3H, d, J = 5.1 Hz, $CH_3$), 0.834 (3H, s, $CH_3$), 1.051 (3H, s, $CH_3$), 1.147 (3H, d, J = 6.9 Hz, $CH_3$), 1.766 (3H, d, J = 6.2 Hz, rha–$CH_3$), 4.959 (1H, d, J = 6.4 Hz, glu–$H_1$), 5.134 (1H, d, J = 7.8 Hz, glu′ –$H_1$), 5.296 (1H, m, olefinic H), 6.252 (1H, s, rha–$H_1$); $^{13}C$ NMR: Tables 1–2.

Fr. 4 was rechromatographed on silica gel with $CHCl_3$–MeOH–$H_2O$ (30: 10: 1), followed on a MCl gel CHP20P column eluting with 70% MeOH to afford 3 (905 mg). IR $\nu_{max}^{KBr}cm^{-1}$: 3460—3420 (OH); FABMS m/z: 939 $[M(C_{46}H_{76}O_{18})+Na]^+$, 923 $[M+Li]^+$, and 909 $[M(C_{45}H_{74}O_{18})+Li]^+$.

Fr. 5 was subjected to a LiChroprep RP–8 column with 70% MeOH as eluent and then on a silica gel column [$CHCl_3$–MeOH–$H_2O$ (25: 10: 1)] to give 4 (2.7 g). IR $\nu_{max}^{KBr}cm^{-1}$: 3460—3420 (OH); FABMS m/z: 1101 $[M(C_{52}H_{86}O_{23})+Na]^+$, and 1087 $[M(C_{51}H_{84}O_{23})+Na]^+$.

**Formation of 3a and 3b.** A soln of 3 (220 mg) and 30% aq. $Me_2CO$ (50 ml) was refluxed for 12 hr to yield 3a as a white powder, mp 195—198°, $[\alpha]_D^{21}$–61.2° (pyridine; c 0.523); $^1H$ NMR$\delta$: 0.895 (3H, s, $CH_3$), 0.975 (3H, d, J = 6.4 Hz, $CH_3$), 1.060 (3H, s, $CH_3$), 1.344 (3H, d, J = 6.4 Hz, $CH_3$), 1.766 (3H, d, J = 5.9 Hz, rha–$CH_3$), 4.974 (1H, d,

J = 6.2 Hz, glu−$H_1$), 5.015 (1H, d, J = 7.2 Hz, glu′ −$H_1$), 5.309 (1H, m, olefinic H), 6.351 (1H, s, rha−$H_1$); $^{13}$C NMR : Tables 1−2. 3 (220 mg) was refluxed in MeOH (40ml) for 10 hr to give 3b as a white powder; mp 190—193 °, $[\alpha]_D^{21}$−73.7 ° (pyridine; c 0.543).

**Formation of 4a and 4b.** 4 was treated as above procedure to provide 4a and 4b. 4a was a white powder; mp 197—199 °, $[\alpha]_D^{21}$−65.8 ° (pyridine; c 0.532); $^1$H NMR: $\delta$ 0.816 (3H, s, $CH_3$), 0.993 (3H, d, J = 6.2 Hz, $CH_3$), 1.040 (3H, s, $CH_3$), 1.195 (3H, d, J = 5.6 Hz, $CH_3$), 1.735 (3H, d, J = 5.9 Hz, rha−$CH_3$), 3.281 (3H, s, $OCH_3$), 4.813 (1H, d, J = 7.6 Hz, glu−$H_1$), 4.924 (1H, glu′ −H), 5.098 (1H, d, J = 6.7 Hz, glu′ ′ −$H_1$), 5.317 (1H, m, olefinic H), 6.193 (1H, s, rha−$H_1$); $^{13}$C NMR: Tables 1−2. Compound 4b was also a white powder; $^1$H NMR: $\delta$ 0.895 (3H, s, $CH_3$), 0.990 (3H, d, J = 6.4 Hz, $CH_3$), 1.045 (3H, s, $CH_3$), 1.317 (3H, d, J = 6.6 Hz, $CH_3$), 1.720 (3H, d, J = 6.0 Hz, rha−$CH_3$), 4.764 (1H, d, J = 6.2 Hz, glu−$H_1$), 4.923, 5.027 (1H each, glu′ −$H_1$, glu′ ′ −$H_1$), 5.317 (1H, br s, olefinic H), 6.152 (1H, s, rha−$H_1$); $^{13}$C NMR: Tables 1−2.

**Acetylation of saponins 1−4.** To each saponin (1−2 mg) was added $Ac_2O$−pyridine (1:1)(0.1 ml) in a micro−tube. After standing at room temp. overnight, the soln was evapd to dryness with $N_2$ and then subjected to EIMS analysis.

**Acid hydrolysis on TLC plate and identification of resulting monosaccharides.** Saponins and prosapogenins were hydrolysed with HCl vapour on a TLC plate (90 ° water bath for 30−50 min. ), followed with $CHCl_3$−MeOH−$H_2O$ (7: 3: 1; lower layer)(9 ml)+HOAc (1 ml) as development and aniline / phthalate as detection for identifying the sugars.

**Partial hydrolysis of 2.** Saponin 2 (145 mg) was suspended in 0.3 N HCl in 70% EtOH (30 ml) and refluxed for 1 hr. The reaction mix. was neutralized with $NaHCO_3$, concd to dryness. The residue was subjected to CC on silica gel with $CHCl_3$−MeOH−$H_2O$ (7: 3: 1; lower layer) to afford 2a as a white powder. mp 280—282 °, $[\alpha]_D^{21}$−69.5 °; $^{13}$C NMR$\delta$: Tables 1−2.

**Enzymatic hydrolysis of 3 and 4.** Compounds 3 (100 mg) and 4 (400 mg ) was each incubated with cellulase in a buffer (NaAc−HOAc; PH 5.0) at 35 ° for 1 hr. The precipitate was collected by filtration and dried. The hydrolysis product of 3 was recrystalized from MeOH to afford 1 as colourless needles. The residue resulted from 4 was chromatographed on silica gel, and elution with $CHCl_3$−MeOH−$H_2O$ (40: 10: 1) afforded 2 and small amount of 1.

Table 1. $^{13}C$ NMR chemical shifts of aglycone moieties in pyridinde–$d_5$ (ppm)

| C | 1 | 2 | 2a | 3a | 4a | 4b |
|---|---|---|---|---|---|---|
| 1 | 37.5 | 37.6 | 37.6 | 37.5 | 37.4 | 37.5 |
| 2 | 30.2 | 30.2 | 30.2 | 30.1 | 30.0 | 30.2 |
| 3 | 78.3 | 78.3 | 78.4 | 78.4 | 78.3 | 78.4 |
| 4 | 39.0 | 39.0 | 39.1 | 38.9 | 38.8 | 39.0 |
| 5 | 141.0 | 140.9 | 140.9 | 140.9 | 140.8 | 140.9 |
| 6 | 121.7 | 121.8 | 121.7 | 121.7 | 121.7 | 121.8 |
| 7 | 32.3 | 32.4 | 32.3 | 32.3 | 32.1 | 32.4 |
| 8 | 31.7 | 31.8 | 31.7 | 31.7 | 31.6 | 31.7 |
| 9 | 50.4 | 50.4 | 50.4 | 50.3 | 50.3 | 50.4 |
| 10 | 37.1 | 37.2 | 37.1 | 37.0 | 37.0 | 37.1 |
| 11 | 21.0 | 21.1 | 21.1 | 21.1 | 21.0 | 21.1 |
| 12 | 39.9 | 39.9 | 39.9 | 39.9 | 39.7 | 40.0 |
| 13 | 40.5 | 40.5 | 40.5 | 40.8 | 40.7 | 40.7 |
| 14 | 56.7 | 56.7 | 56.7 | 56.6 | 56.5 | 56.6 |
| 15 | 32.2 | 32.3 | 32.2 | 32.4 | 32.2 | 32.4 |
| 16 | 81.1 | 81.1 | 81.1 | 81.1 | 81.2 | 81.1 |
| 17 | 62.9 | 63.0 | 63.0 | 63.7 | 64.1 | 63.8 |
| 18 | 16.3 | 16.3 | 16.3 | 16.3 | 16.2 | 16.3 |
| 19 | 19.4 | 19.4 | 19.4 | 19.4 | 19.3 | 19.4 |
| 20 | 42.0 | 42.0 | 42.0 | 40.6 | 40.4 | 40.7 |
| 21 | 15.0 | 15.0 | 15.0 | 16.4 | 16.2 | 16.4 |
| 22 | 109.3 | 109.3 | 109.3 | 110.7 | 112.6 | 110.7 |
| 23 | 31.8 | 31.9 | 31.8 | 37.1 | 30.7 | 37.1 |
| 24 | 29.2 | 29.3 | 29.3 | 28.2 | 28.1 | 28.3 |
| 25 | 30.5 | 30.6 | 30.6 | 34.2 | 34.1 | 34.2 |
| 26 | 66.9 | 66.9 | 66.9 | 75.2 | 75.1 | 75.2 |
| 27 | 17.3 | 17.3 | 17.3 | 17.4 | 17.1 | 17.4 |
| $OCH_3$ | | | | | 47.3 | |

Table 2. $^{13}C$ NMR chemical shifts of sugar moieties in pyridine–$d_5$ (ppm)

| | 1 | 2 | 2a | 3a | 4a | 4b |
|---|---|---|---|---|---|---|
| glu–1 | 100.4 | 100.2 | 102.4 | 100.3 | 99.9 | 100.1 |
| 2 | 79.4 | 78.4 | 74.8 | 79.4 | 78.2 | 78.0 |
| 3 | 78.0 | 76.2 | 76.8 | 77.9 | 76.0 | 76.1 |
| 4 | 71.8 | 82.1 | 81.3 | 71.8 | 81.7 | 81.9 |
| 5 | 78.1 | 77.4 | 76.4 | 78.0 | 77.3 | 77.5 |
| 6 | 62.7 | 62.1 | 62.3 | 62.6 | 61.8 | 61.9 |
| rha–1 | 101.9 | 101.8 | | 101.9 | 101.6 | 101.7 |
| 2 | 72.3 | 72.4 | | 72.3 | 72.2 | 72.3 |
| 3 | 72.9 | 72.8 | | 72.7 | 72.6 | 72.7 |
| 4 | 74.0 | 74.2 | | 74.0 | 73.9 | 74.1 |
| 5 | 69.4 | 69.4 | | 69.4 | 69.3 | 69.4 |
| 6 | 18.5 | 18.6 | | 18.5 | 18.5 | 18.6 |
| glu–1 | | 105.1 | 104.9 | | 104.9 | 105.0 |
| 2 | | 75.0 | 74.9 | | 74.7 | 75.1 |
| 3 | | 78.3 | 78.4 | | 78.1 | 78.3 |
| 4 | | 71.4 | 71.6 | | 71.1 | 71.3 |
| 5 | | 77.7 | 78.2 | | 77.5 | 77.6 |
| 6 | | 62.2 | 62.7 | | 62.0 | 62.2 |
| 26–O– | | | | | | |
| glu–1 | | | | 104.7 | 104.7 | 104.8 |
| 2 | | | | 75.0 | 74.9 | 75.2 |
| 3 | | | | 78.3 | 78.2 | 78.2 |
| 4 | | | | 71.6 | 71.6 | 71.7 |
| 5 | | | | 78.2 | 78.0 | 78.1 |
| 6 | | | | 62.7 | 62.7 | 62.8 |

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